

DNA 7500 ASSAY PROTOCOL: AGILENT TECHNOLOGIES®

This protocol is adapted from an Agilent Technologies protocol by the Gene Expression Lab. *For additional technical inquiries, contact Technical Service at 800-227-9770 or www.agilent.com/chem/labonachip*

BEFORE STARTING THE EXPERIMENT

REAL TIME ASSAY PROTOCOL

Step A. Prepare Chip

Step B. Load chip onto Bioanalyzer

EXAMPLE OF DATA ACQUISITION AND ANALYSIS

TROUBLESHOOTING

BEFORE STARTING THE EXPERIMENT

Prepare samples to add to chip:

- Label appropriate number Eppendorf tubes
- Add 5µl of Marker to each tube (add to ladder tube 1st)
- Add 1µl of Sample to appropriate tube (add ladder tube 1st)
- Vortex, spin briefly

DNA 7500 ASSAY PROTOCOL

STEP A: Prepare Chip

- Take out chip cleaner
- Add 350µl DEPC treated H₂O to chip cleaner
- Put chip cleaner on Bioanalyzer for ~10 seconds
- Remove chip cleaner and allow Bioanalyzer to air dry for ~ 10seconds
- Close Bioanalyzer
- Start up Agilent Technologies program on computer
- Select Assay
 - dsDNA
 - DNA7500
- Take out Chip
- Add 9µl of gel dye mix to the well marked **G**
- Put on docking station
- Close station and press plunger (30 seconds)

- Release clip and help pull syringe up
- Release other lock
- Add 9µl of gel dye to the wells marked **G**
- Add 6µl of ladder and samples to their appropriate wells

** If do not have 12 samples, must load the empty wells with 6µl marker.

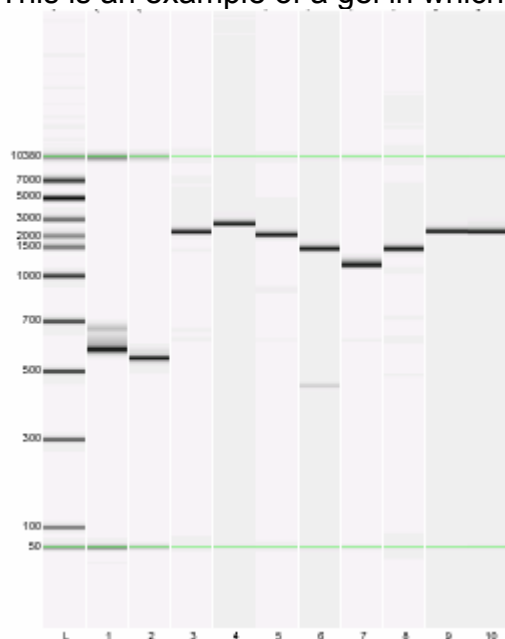
STEP B: Load chip

- Load chip into Bioanalyzer
- Click Start
- Enter the # of samples
- Hit START
- Label the Samples on the computer

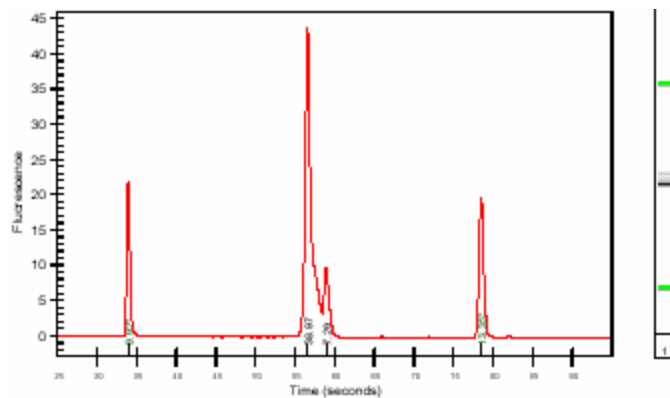
**Takes ~30 minutes

EXAMPLE OF DATA ACQUISITION AND ANALYSIS

This is an example of a gel in which we ran ten samples.



Once each well has been run, an electropherogram shows the peaks in each well. In addition, the Results table for each well is available for gel analysis.



| Peak | Mid Time (secs) | Area | Size (bp) | Conc. (ng/ul) | Molarity (nmol/l) | Observations |
|------|-----------------|-------|-----------|---------------|-------------------|--------------|
| 1* | 33.95 | 0.02 | 50 | 8.3 | 251.51 | Lower Marker |
| 2 | 58.95 | 38.97 | 589 | 11.5 | 29.44 | |
| 3 | 58.95 | 7.29 | 872 | 2.1 | 4.74 | |
| 4* | 78.55 | 13.35 | 10390 | 4.2 | 0.61 | Upper Marker |

TROUBLESHOOTING

- A. The gel results show a well is completely dark
 1. There are several possible causes of this. Too much DNA could have been used. For the DNA 7500 it is recommended that the DNA concentration be between 0.5-50ng/ul.
 2. There could have been an air bubble that prevented accurate reading of the well. To prevent this make sure to insert the tip of the pipette to the bottom of the well when dispensing.
- B. There is a peak, but the Results Tables do not show the peak or its measurements.
 1. To make a small peak recognized by the computer, the settings must be changed so that the min. peak height is smaller. The default setting is 8.0. This can be changed to a smaller area so that the peak will be recognized and the concentration and size are given.